ARIA

HindIII Digestion Protocol

iGEM upf. prbb arquicalcul TASALIA SOCIEDAD DE TASACIÓN

HindIII digestion

Introduction

To achieve our goal of performing an in vitro transcription, we decided to use the HindIII digestion. We followed the instructions of the in vitro transcription kit from Thermofisher.

Materials

- Cuts smart
- Hind III
- EDTA
- Na acetate
- Ethanol

Procedure

Linearization with Hind III Digestion restriction enzyme

1. **Linearization** with **Hind III Digestion** restriction enzyme downstream of the insert to be transcribed.
    1. **gRNA plasmids** using Hind III choose 2 plasmids
    2. **Reaction**:
    - 17 uL plasmid
    - 2 uL cuts smart
    - 1 uL Hind III
    - Total Solution = 20 uL
2. **Terminate** restriction digest
    1. **Add** 1/20th volume 0.5 M **EDTA**
    2. 1/10th volume of 3 M **Na acetate** or 5 MNH4 acetate
    3. 2 volumes of **Ethanol**
    4. **Mix well** and chill at –20°C for at least 15 min. Then **pellet** the DNA for 15 min in a microcentrifuge at top speed.
    5. **Remove** the supernatant, re-spin the tube for a few seconds, and remove the residual fluid with a very fine-tipped pipet.
    6. **Resuspend** in dH2O or TE buffer at a concentration of 0.5–1 µg/µL.
3. **Run a gel**

Bibliography

1. (N.d.-f). Retrieved October 19, 2021, from Thermofisher.com website: https://tools.thermofisher.com/content/sfs/manuals/1330M_G.pdf